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14. ABSTRACT

Tinnitus is a common auditory perceptual disorder whose neural substrates are under intense debate. This project takes a multimodal imaging approach to dissect its neural circuits. Resting-state functional MRI revealed increased striatal-auditory cortical connectivity was isolated to area LC, positioned at the junction of the head and body of the caudate nucleus, where hyperconnectivity was positively correlated with tinnitus severity. Striatal connectivity was also increased between area LC and dorsal prefrontal cortex, but reduced between area LC and nucleus accumbens of the ventral striatum. Connectivity of primary auditory cortex was increased to regions within the default-mode network. Those findings provide further evidence to support a striatal gating model of tinnitus, where dysfunctionally-permissive area LC enables auditory phantoms to reach perceptual awareness. Magnetoencephalographic resting-state functional connectivity imaging (MEGI) in tinnitus subjects in the alpha-band showed increased medial prefrontal cortical connectivity. MR spectroscopic imaging (MRSI) successfully captured GABA spectroscopic levels in the caudate nucleus and auditory cortices reliably. 7T structural MRI delivered higher resolution of anatomic structures of the basal ganglia and auditory cortex.

15. SUBJECT TERMS

Tinnitus; Multimodal Imaging; fMRI; Magnetoencephalographic Imaging; MR Spectroscopic Imaging; Tinnitus Handicap

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1. INTRODUCTION:

The overall objective of this research project is to test specific predictions that emerge from a novel basal ganglia-centric tinnitus model by evaluating functional connectivity of the striatum to related auditory brain structures and of neural oscillations in auditory cortex, neurotransmitter (Glutamate and GABA) expression levels in the striatum and auditory cortex, and morphologic microstructure of the dorsal and ventral striatum, in three human cohorts:

COHORT 1 - mild to moderate high frequency hearing loss with tinnitus
COHORT 2 - mild to moderate high frequency hearing loss without tinnitus
COHORT 3 - normal to mild high frequency hearing loss without tinnitus

We plan 3T functional magnetic resonance imaging, magnetoencephalography imaging, 7T magnetic resonance spectroscopic imaging, and 7T structural MRI.

2. KEYWORDS:

Tinnitus; Multimodal Imaging; fMRI; Magnetoencephalographic Imaging; MR Spectroscopic Imaging; Tinnitus Handicap Inventory.

3. ACCOMPLISHMENTS:

What were the major goals of the project?:

Specific Aims (SA)

Specific aim 1 will assess basal ganglia and auditory cortical functional connectivity in tinnitus and its association with level of distress using resting-state fMRI.

SA1a: To determine if the dorsal striatum has abnormal functional connectivity with auditory cortex in tinnitus.
SA1b: To determine if the ventral striatum has abnormal functional connectivity to limbic structures that is related to tinnitus distress.

This aim is 50% complete. We have a manuscript under review in the Journal of Neuroscience.

Specific aim 2 will examine the profile of functional connectivity of auditory cortical oscillations with the rest of the brain in tinnitus using MEGI.

> SA2a: To determine if functional connectivity relationships of neural oscillations in auditory cortex are abnormal in tinnitus.

This aim is 50% complete. We have completed half of our recruitment target, and are analyzing this data set.

Specific aim 3 will assess the balance of neurotransmitter levels of the basal ganglia and auditory cortex using MRSI and the microstructure of the basal ganglia using structural MRI in tinnitus.

> SA3a: To determine if the striatum and auditory cortex have an abnormal balance of excitation and inhibition in tinnitus by measuring GABA and Glutamate levels.
> SA3b: To determine if the microstructure of the dorsal or ventral striatum is abnormal in tinnitus.

This aim is 30% complete. We have started data collection in tinnitus and control subjects, and improved data quality and reliability by refining MR acquisition sequences.

What was accomplished under these goals?

Specific aim 1:

We have completed one study of resting-state fMRI comparing chronic tinnitus and control subjects. This paper is now under review in the Journal of Neuroscience. In this paper we show that chronic tinnitus subjects have increased functional connectivity between the caudate nucleus and auditory cortices. This increased functional connectivity is correlated with tinnitus severity. The provocative result provides strong support for our striatal model of tinnitus.

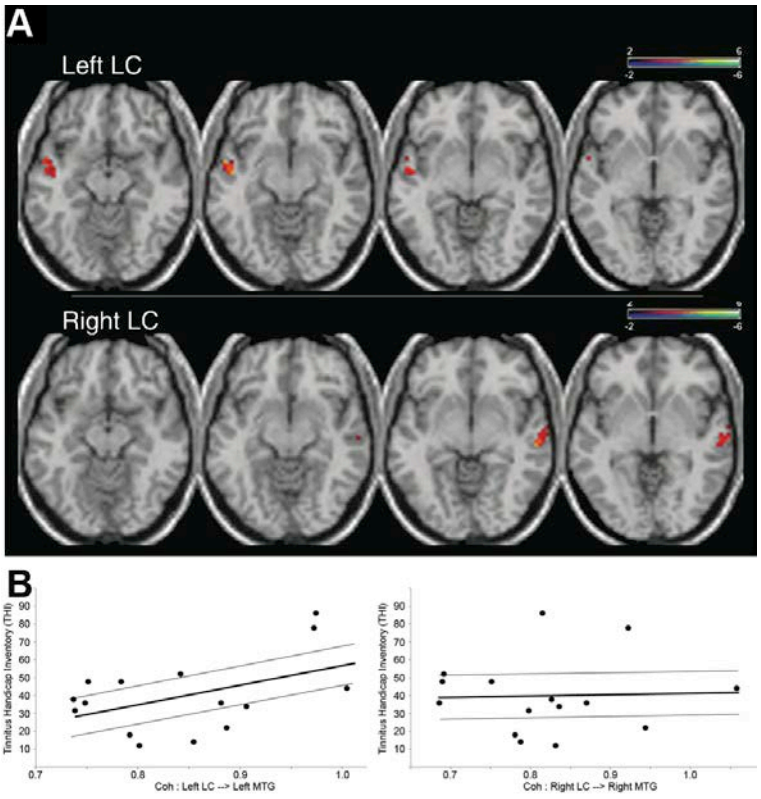


Figure 1. Comparison of Tinnitus vs Control: Area LC (caudate nucleus) seeds.

A. Between-group analysis (unpaired t-test; cluster threshold $p<0.005$ $k=40$) shows differences between patients with chronic tinnitus and matched controls for connections of area LC (LC) of the caudate nucleus with regions in the temporal lobe. Top panel: group differences for left LC. Bottom panel: group differences for right LC. Increases in resting-state connectivity in chronic tinnitus (red) are identified between LC and auditory regions of the superior temporal gyrus in the ipsilateral hemisphere.

B. Correlation between THI and coherence magnitude of area LC with MTG for left LC seeds (top row) and right LC seeds (bottom row). THI – Tinnitus Handicap Inventory.

Next year, we will complete data collection in a second cohort of tinnitus subjects using both 3T and 7T MR technologies to detail and extend our initial findings, and examine reliability of correlations between imaging and behavioral measurements.

Specific aim 2:

We have completed data collection and analysis in one set of tinnitus and control subjects using magnetoencephalographic imaging (MEGI). Manuscript preparation is in progress.

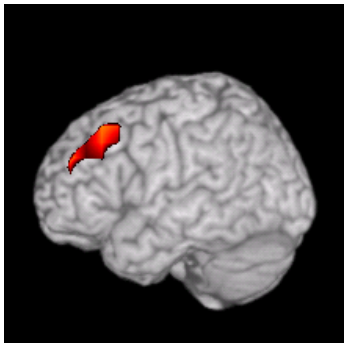


Figure 2. Resting-state alpha-band (10Hz) MEGI functional connectivity in tinnitus patients compared to healthy controls.

Tinnitus subjects show increased functional connectivity in medial frontal cortex, thresholded and corrected for multiple comparisons (false discovery rate of 5%). As highlighted by this image, MEGI is complementary to resting-state fMRI and demonstrates abnormal neural oscillatory network connectivity in tinnitus.

For year 2 of this award, we will continue to collect MEGI data in a second set of tinnitus subjects and controls, both with mild-to-moderate high frequency sensorineural hearing loss. We will also be analyzing cortical response latencies and amplitudes to pure tones in the MEGI data.

Specific aim 3:

We have begun collecting GABA MR-Spectroscopy data in tinnitus and control subjects in 7T MRI. We have refined our procedures for collection of GABA data in caudate nucleus and auditory cortex. We have analyzable data in 5 tinnitus subjects, and will be ramping up data collection in 7T this coming year. We have also collected high-resolution 7T structural MRI data that enables us to perform quantitative morphometric analyses.

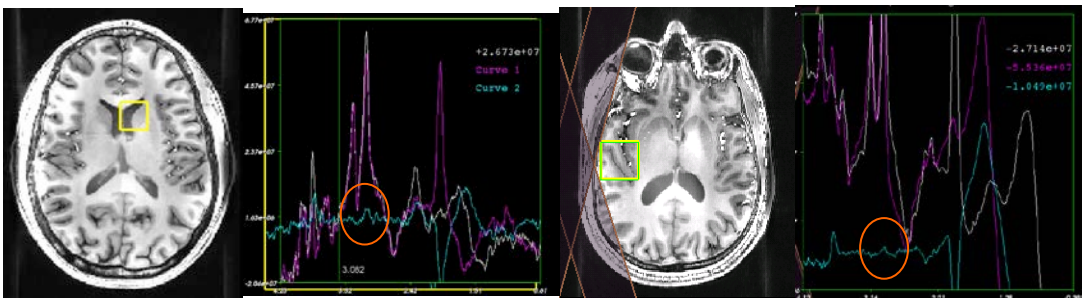


Figure 3. 7T GABA MR-Spectroscopic imaging peak extracted from right caudate (left two panels) and left auditory cortex (third and fourth panels) from a *control* subject. The GABA peak is marked by an orange oval.

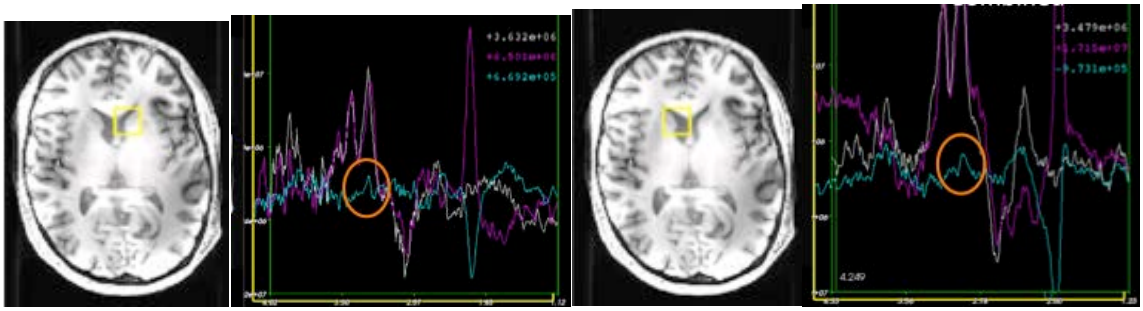


Figure 4. 7T GABA MR-Spectroscopic imaging peak extracted from right caudate (left two panels) and left caudate (third and fourth panels) from a *tinnitus* subject. GABA signal identified in both hemispheres has higher amplitude. The GABA peak is marked by an orange oval.

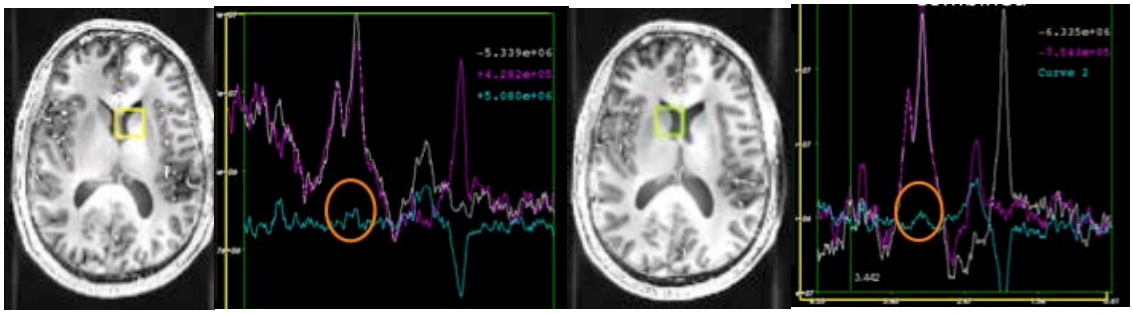


Figure 5. 7T GABA-MR Spectroscopic imaging peak extracted from right caudate (left two panels) and left caudate (third and fourth panels) from another *tinnitus* subject. Again, GABA signal is identifiable in both hemispheres. The GABA peak is marked by an orange oval.

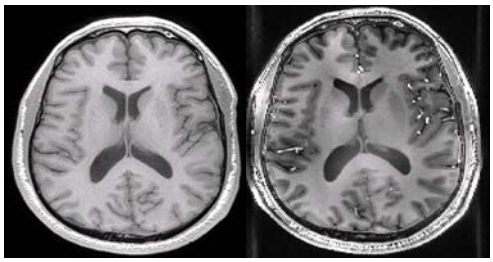
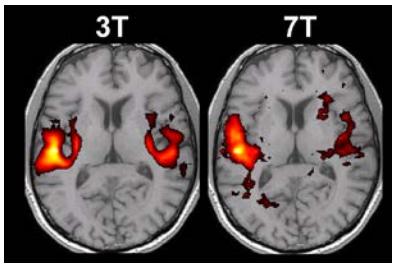
A.  B. 

Figure 6. Comparison of structural and functional data: 3T vs. 7T.

A. 3T (left) and 7T (right) structural MRI in the same tinnitus subject. An axial slice is shown across the caudate nucleus. Microvasculature and other details can be seen at 7T, but not at 3T.

B. Resting-state fMRI in the same tinnitus subject obtained at 3T and 7T, where the seed was placed at primary auditory cortex. 7T provides superior definition of spatial and connectivity structures.

Early data MR spectroscopic data are supportive of our initial hypothesis of abnormal GABA expression in basal ganglia and auditory cortex in tinnitus. We plan to complete this specific aim 3 of the study in years 2 and 3.

What opportunities for training and professional development has the project provided?

The project has provided training and professional development for several members of the study team. Two research associates, Ms. Danielle Mizuiri and Mr. Garrett Coleman, are actively engaged in optimizing subject recruitment and imaging data collection (MRI and MEGI). We have also trained Otolaryngology-Head and Neck Surgery clinicians (Dr. Seth Pross, UCSF Chief Resident) and (Dr. Jolie Chang, UCSF Assistant Professor) on tinnitus imaging research. Based partly on this experience, the Dr. Pross has accepted a fellowship position at Johns Hopkins University to further his study of Neurotology or neurological diseases of the ear. Dr. Chang was awarded a Junior Faculty Development Award from the Triological Society to support MEGI studies on hearing disorders. A junior scientist from UCSF Audiology, Jennifer Henderson-Sabes, has joined the study team to accelerate subject accrual. Three postdoctoral junior scientists have been trained on fMRI, MRSI and MEGI data acquisition and analysis. Dr. Leighton Hinkley is a postdoctoral fellow who has been leading the analysis of resting-state fMRI data in 3T and 7T scanners. Dr. Yan Li is a staff scientist who is developing MR acquisition sequences for GABA-MRSI in 7T MR scanners. Dr. Carly Demopoulos is a postdoctoral fellow who is leading the analysis of resting-state MEGI. She is also a certified neuropsychologist who is involved in

screening and behavioral assessment of all study subjects. Those three junior scientists work closely with other postdoctoral fellows on data integration across multiple imaging modalities and behavioral assessments. All team members are supervised by the the PI and Co-Investigator, Dr. Srikantan Nagarajan, to ensure appropriate progress on all aspects of the project. The study team holds monthly meetings to assess recruitment status, imaging results, and information dissemination. There are weekly seminars and journal clubs for scientists on the team to enrich intellectual development. In the coming year, as we publicized results from our project, study team scientists will have the opportunity to travel to international conferences such as those held by the Society for Neuroscience and the Organization for Human Brain Mapping to disseminate information.

How were the results disseminated to communities of interest?

Our first paper is currently under review in the Journal of Neuroscience. We are planning to submit the first MEGI paper by the spring 2015, and a third paper on the MRSI findings by year 3. In the coming year, as we present the results from our project, scientists will have the opportunity to travel to international conferences such as those held by the Society for Neuroscience and the Organization for Human Brain Mapping to disseminate information.

What do you plan to do during the next reporting period to accomplish the goals?

Our plans for the next reporting period include accelerated recruitment and imaging of tinnitus and control subjects using the following imaging modalities – 3T fMRI, MEGI, 7T fMRI, and 7T structural MRI. We will complete analysis of the MEGI data for a manuscript preparation in this period. MRSI data collection and manuscript completion is planned for year 3.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them:

Subject recruitment preference for Veterans proved somewhat challenging to fulfill. We encountered a surprisingly high frequency of Veterans with disqualifying factors: moderate or more severe PTSD, metal in the head, and cardiac stents (7T fMRI and 7T MR spectroscopic imaging contraindicated). Resolution of subject recruitment delay will be addressed by reorienting efforts to capture tinnitus patients from UCSF Audiology, Bay Area Kaiser Permanente, and sizeable private practice clinics in year 2.

Changes that had a significant impact on expenditures:

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Steven W. Cheung
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.74
Contribution to Project:	Dr. Cheung supervises all aspects of the study. He is actively engaged in audiological and imaging activities associated with this project. Dr. Cheung will jointly analyze data and prepare manuscripts with the research team. Dr. Cheung co-discovered area LC, the dorsal striatal structure hypothesized to be important in tinnitus awareness.

Name:	Srikantan S. Nagarajan
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.98
Contribution to Project:	Dr. Nagarajan supervises training of postdoctoral fellows to analyze resting-state MEGI, fMRI and MRSI data. Dr. Nagarajan is Director of the UCSF Biomagnetic Laboratiory and is very experienced in functional connectivity imaging studies in humans.

Name:	Caroline Racine Belkoura
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.20
Contribution to Project:	Dr. Belboura characterizes subjects neuropsychologically. She is an experienced neuropsychologist with specific expertise in performing neurocognitive evaluations. Dr. Racine performs, scores, analyzes, and interprets all neuropsychological tests.

Name:	Carly Demopoulos
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6.0
Contribution to Project:	Dr. Demopoulos is a postdoctoral fellow and a certified neuropsychologist who is involved in screening and behavioral assessment of all study subjects. She leads the analysis of resting-state MEGI.

Name:	Leighton B. Hinkley
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6.0
Contribution to Project:	Dr. Hinkley is a postdoctoral fellow. He leads the analysis of resting-state fMRI.

Name:	Danielle Mizuiri
Project Role:	Study Coordinator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6.0
Contribution to Project:	IRB management, subject recruitment, and imaging data collection (MRI and MEGI).

Name:	Coleman Garrett
Project Role:	Study Coordinator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6.0
Contribution to Project:	Along with Ms. Mizuiri, Mr. Garret is engaged in IRB management, subject recruitment, and imaging data collection (MRI and MEGI).

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS:

Not applicable.

9. APPENDICES:

Nothing to report.